SUPPLEMENTARY FIGURE LEGENDS.

Supplementary Figure 1. BAPTA-AM and W-7 regulate EF-2 phosphorylation in Neo and HA-Pnck HEK-293 cells. Both Neo (lanes 1-2, 5-6, 9-10 and 13-14) and HA-Pnck HEK-293 cells (lanes 3-4, 7-8, 11-12 and 15-16) were plated at low cell density, serum starved and incubated without (Vehicle/DMSO, lanes 1-4 and 9-12) or with 10 μM BAPTA-AM (lanes 5-8) or with 30 μM W-7 (lanes 13-16) overnight followed by stimulated without (lanes 1, 3, 5, 7, 9, 11, 13 and 15) or with (lanes 2, 4, 6, 8, 10, 12, 14 and 16) 10 nM EGF for 5 min. Equal amounts of total protein containing lysates were immunoblotted by Phospho-EF-2 (Thr-56) (WB: P-EF-2), anti-HA mAb (WB: HA-Pnck) and MAPK (WB: MAPK).

Supplementary Figure 2. Mass spectrometric analysis of Hsp90 immunoprecipitated from HA-Pnck HEK-293 cells.

(A) MS/MS fragmentation spectra for the associated peptide sequences of precursor ions of specific phosphorylated peptides derived from HSP90 α and β (Accession no. P07900 and P08238 respectively). The doubly charged precursor ion at *m/z* 656.74 of the modified peptide spanning residues 613 to 623 showed a 79.9 amu shift that could be accounted for by phosphorylation at the threonine residue at position 616. The singly charged b ions from b1 to b3 were identical to those of the unmodified peptide. However the mass difference between b4 and b3 was 181.02 daltons which can be explained by the presence of phosphorylation at that site. (B) The product ion spectrum of the doubly charged modified peptide with a m/z 691.36 spanning residues 83 to 95 of Hsp 90 β (Accession no. P08238) showed a y7 ion at m/z 739.33 which could be accounted for by mass shift of 79.9 daltons thus indicating the phosphorylation of the threonine residue at Thr 89. The Hsp90 α peptide is identical to Hsp90 β peptide except that the fourth amino acid is isoleucine (I). (C) The peptide with the sequence VVDSEDLPLNISR spanning amino acid residues from 388 to 400 of Hsp90 α (Accession no. P07900) displayed a doubly charged ion with *m/z* 522.9 in the total ion chromatogram. The precursor molecular mass showed a 79.9 dalton shift due to phosphorylation of serine at position four in the peptide. The product ion spectrum of this modified peptide showed a b4 ion at m/z

497.16 which corresponded to a mass difference of 167 daltons with respect to b3 ion (m/z 330.16). This mass difference observed between the b4 and b3 ions corresponds to the modified serine at position 391 in HSP90α. This mass corresponds to a serine (87.08 Da) plus an additional mass of 79.9 Da, thus confirming the location of the modified residue.

Supplementary Figure 3. Pnck downregulates EGFR expression. Equal amount of total protein from HEK-293 cells stably expressing Neo (lane 1) or HA-Pnck (lane 2) were immunoblotted with anti-EGFR mAb, Ab-15 (NM) (panel A), anti-EGFR mAb, Ab-1, clone 528 (NM) (panel B), anti-EGFR pAb (1005) (SC) (panel C) and anti-Pnck antibodies (panel D).